Identity Gene Expression in Proteus Mirabilis

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Karine A. Gibbs[1], Larissa M. Wenren[1] and E. Peter Greenberg[2]

[1]Department of Molecular and Cellular Biology, 16 Divinity Street,
Harvard University, Cambridge, MA 02138

[2]Department of Microbiology, Box 357242, 1705 NE Pacific Street,
University of Washington School of Medicine, Seattle, WA 98195-7242

Running title: *P. mirabilis* identity gene expression

Corresponding author: E. Peter Greenberg
Department of Microbiology
HSB-K359
1705 NE Pacific Street
Box 357242
University of Washington
Seattle, WA 98195-7242
Phone: 206.616.2881
FAX: 206.543.8297
Email: epgreen@uw.edu

Abstract

Swarming colonies of independent *Proteus mirabilis* isolates recognize each other as foreign and do not merge together whereas apposing swarms of clonal isolates merge with each other. Swarms of mutants with deletions in the *ids* gene cluster do not merge with their parent. Thus *ids* genes are involved in the ability of *P. mirabilis* to distinguish self from non-self. Here we have characterized expression of the *ids* genes. We show that *idsABCDE* and *F* are transcribed as an operon, and we define the promoter region upstream of *idsA* by deletion analysis. Expression of the *ids* operon increased in late logarithmic and early stationary phase and appeared to be bistable. Approaching swarms of non-self populations led to increased *ids* expression and increased the abundance of *ids*-expressing cells in the bimodal population. This information on *ids* gene expression provides a foundation for further understanding the molecular details of self-non-self discrimination in *P. mirabilis*.

Introduction

The γ–Protobacterium *Proteus mirabilis* is a leading cause of recurrent urinary tract infections and displays some extraordinary behaviors (15). *P. mirabilis* undergoes a morphologically distinct developmental cycle during growth on surfaces (reviewed in 8, 15, 18). Upon contact with a surface, flagellated rod-shaped swimmer cells (1-2 μm in length) differentiate into hyper-flagellated, polynucleoid, elongated cells (10-80 μm in length). Colonies can swarm across surfaces rapidly and the elongated cells, called swarmer cells, do not divide during migration. Expression of many virulence genes appears to be linked to swarmer cell differentiation, and there is a distinction between genes expressed during liquid growth versus those expressed during surface-associated growth (1, 2, 5, 6, 16, 18). The migrating front of a swarm will periodically arrest movement; cells will dedifferentiate to short cells and consolidate through cell division. After another round of differentiation, swarmer cells will migrate forward once again. This behavior results in colonies with a bulls-eye-like pattern (reviewed in 8, 18).

Of particular interest to us, a boundary will form between approaching swarms of different strains but not between two swarms of a single strain (Fig. 1A). The boundary formation demonstrates that *P. mirabilis* populations have an ability to distinguish self from non-self. We recently identified a gene cluster, *idsABCDEF*, involved in *P. mirabilis* self versus non-self recognition (10). Swarms of mutants in the *ids* gene cluster form a boundary with their parent. Two of the genes, *idsD* and *idsE*, encode functions for determining strain-specific identity. Of the remaining genes, *idsB*, *idsC*, and *idsF* are required for self versus non-self recognition but are not identity determinants. The *idsA* gene is not required for recognition, but it has been termed an

ids gene because *idsA* is possibly co-transcribed with *idsB-F*. There is no available information on *ids* gene expression.

Bacterial colonies can be considered either as populations of cells or as populations of individual members of a species. The ability to distinguish self from non-self is a behavior conserved in both cellular and organismal population dynamics. Territoriality, a social behavior, is conserved among many organisms and is often driven by competition for resources, competition for mates, or the need to segregate genetic pools (22). We view swarm colony boundary formation as an example of territoriality. Because we have begun to identify genetic determinants for the self-recognition required for this territoriality, we believe *P. mirabilis* can serve as a model to help understand the molecular mechanisms of recognition and territoriality and can provide insights about their evolution. To better develop this model, we initiated a study of *ids* gene expression. Here we show that *idsABCDEF* constitute an operon; that expression of this operon results in a bimodal pattern consistent with bistability; and that this expression positively correlates with increasing cell densities. We also provide evidence that *ids* gene expression is altered by an approaching swarm of a strain recognized as non-self.

Materials and Methods

Bacterial strains and growth media. We used *Escherichia coli* Top10 (Invitrogen), *E. coli* S17-1λpir (7), *P. mirabilis* BB2000 (3), *P. mirabilis* Δ*ids* (10), and *P. mirabilis* BB2000 constitutively expressing *gfp* (10). We used LB-Lennox (LB) broth supplemented with 1 mM MOPS buffer for growth of *P. mirabilis* swimmer cells. The media for *P. mirabilis* colonial growth were either CM55 Blood Agar Base agar (Remel Inc., Lenexa, KS) for swarm colony

growth or Low Swarm (LSW^-) agar for isolation of single colonies (3). *E. coli* was grown in LB broth and on LB agar. Antibiotics were used as follows: 35 μg/ml kanamycin (Km); 100 μg/ml rifampicin; and chloramphenicol at 35 μg/ml for *E. coli* and 75 μg/ml for *P. mirabilis*. All media contained antibiotics appropriate for selection or maintenance of plasmids.

Plasmid constructions. Our *idsA* promoter reporter vector pKG100 was constructed by digesting pids_{BB}Δ*ABC* (10) with NheI and HincII, and ligating the *ids* promoter-containing digestion product to pPROBE-GFP-AT digested with SpeI and EcoRV (14). The resulting pKG100 construct contains *idsA* bp -788 to +11 fused to *gfp*. To construct the promoterless *gfp* control vector, pKG101, *gfp* was PCR amplified from pPROBE-GFP-AT by using a primer complementary to the first nine codons of *gfp* and an NheI restriction site tail and a primer complementary to the last nine codons of *gfp* with an AgeI restriction site tail. The PCR product was digested with NheI and AgeI and ligated to similarly digested pBBR1-NheI (10) to yield pKG101. The *idsA-gfp* fusion vector pKG102 was constructed from a PCR product consisting of the 788-bp region upstream of *idsA* and the first three codons of *idsA* amplified from pids_{BB} (10). The PCR primers were designed to have NheI and SacI restriction site tails. After restriction enzyme treatment the PCR product was ligated to NheI and SacI-digested pKG101 to form pKG102, which contained *gfp* fused in-frame with the first nine codons of *idsA* and the *idsA* upstream promoter region. To test whether the DNA between the *idsA* and *B* coding regions exhibited detectable promoter activity, we generated pKG103, which contained the complete 72-bp intergenic region and the first 3 codons of *idsB* fused in-frame with *gfp* from pKG101. As above, the PCR product was generated using primers to the desired region with NheI and SacI restriction site tails. The PCR product was ligated into NheI-SacI-digested pKG101 to form

pKG103. We constructed pKG104, a pKG100-deletion derivative by removal of bp -788 to -435 with respect to the *idsA* translation start site via excision of the 353-bp NheI-XbaI fragment.

In all cases plasmids were isolated from ligation mixtures by transformation of *E. coli* Top10. Transformants were selected on Km-agar plates. Constructs were confirmed by DNA sequencing of the inserted region (SeqWright DNA Technology Services, Houston, TX). Plasmids were moved to *E. coli* S17-1λpir by transformation and then moved from *E. coli* S17-1λpir to *P. mirabilis* BB2000 by conjugation. Transconjugants were selected on LSW$^-$ plates containing Km.

Reverse-transcription polymerase chain reaction. Wild-type *P. mirabilis* strain BB2000 was grown to late logarithmic phase in LB broth. Cells were harvested and stored at -20°C. After RNA extraction contaminating DNA was digested with RQ DNase (Promega Co., Madison, WI) and removed by using an RNeasy kit and the RNA Clean-Up protocol (Qiagen Inc., Valencia, CA). cDNA was generated by using a SuperScript III First Strand Synthesis Kit (Invitrogen Co., Carlsbad, CA) with either random hexamer or Oligo dT primers. Reactions in which reverse transcriptase was omitted served as controls for DNA contamination. PCR was performed by using Taq polymerase (Invitrogen Co.) and gene-specific primers. Samples were isolated after 35 cycles and examined by agarose gel electrophoresis.

Imaging swarming *P. mirabilis* colonies. CM55 agar plates were inoculated from stationary phase cultures with an inoculation needle at a spacing of approximately 2 cm. Images were captured on an Optio W10 digital camera (Pentax Imaging Company, Westminster, CO) after overnight incubation at 37°C. False-colored microscope images were prepared by overlaying the

fluorescence channel as green and the phase channel as red in Adobe Photoshop CS5 (Adobe Systems, San Jose, CA). Image contrast was equally increased across the entire raw image, and images were then cropped to size. Microscopy was as described previously (10).

Fluorescence measurements. For liquid cultures, cells were grown aerobically in LB-Lennox broth at 37°C. Cells were harvested by centrifugation, washed and then suspended in PBS. For cells isolated from swarms, *P. mirabilis* was permitted to form swarms on CM55 blood agar media overnight at 37°C as described above. Cells were scraped from colonies with a wooden dowel and suspended in 500 µl PBS. The cell suspension was centrifuged, the cell pellet washed with PBS, and finally suspended in 500 µl PBS. Samples (200 µl) were dispensed into wells of a sterile Costar 96-well plate (Corning Inc., Lowell, MA). The optical density at 595 nm (for culture density) and fluorescence at 485/535 nm in each well was measured by using a Tecan GENios Pro-Basic microplate reader (Tecan Group Ltd., Männedorf, Switzerland). Fluorescence measurements of an entire swarm were performed in Costar 6-well plates. All swarm-well data consist of at least three experiments. Each well contained 5 ml of CM55 agar. Where indicated we placed a sterile 0.22-µm filter across the middle of the well. Agar-containing wells were inoculated as described above. After an overnight incubation at 37°C, absorbance and fluorescence in wells was measure as described above. Background fluorescence (uninoculated medium) was subtracted and the data are presented as fluorescence normalized to absorbance.

Microscopy. Microscopy of swarms was performed as described previously (10). Cells grown in broth were harvested, washed with PBS, mounted onto glass slides, and imaged immediately. We measured fluorescence intensity by using MetaMorph 6.3r2 (Molecular Devices, Sunnyvale,

CA), and subsequent analysis was with Microsoft Excel 2004 (Microsoft Co., Redmond, WA), GraphPad Prism 5.0b (GraphPad Software, Inc., La Jolla, CA). For analysis of single cells from broth cultures, the Integrated Morphometry Analysis function of MetaMorph was used to identify and measure the physical dimensions of individual cells in phase images. The average, minimum, and maximum intensities of each identified cell were then measured from fluorescence images. The slide background fluorescence intensity was subtracted from the total cellular intensity. The average intensities of individual cells were sorted to study the variation in *idsA-gfp* expression from cell to cell. For image analysis in swarms, we used the Linescan function in MetaMorph to measure the average and maximum intensity of pixels from one point to another across the image, specifically from a region inside the swarm of the reporter strain, across the boundary or merger region, and into the swarm of the apposing strain. Each recorded pixel intensity consisted of the mean, or maximum, intensity of a 1-pixel wide by 20-pixel tall slice, which corresponded to a 0.65-µm by 13-µm slice through a swarm. We report the averages from multiple images.

Results

The *idsA-F* genes constitute an operon. The organization of *idsA-F* and the flanking genes is shown in figure 1B. Our previous studies showed that *idsB, C, D, E* and *F* were involved in self vs non-self recognition but *idsA* mutants did not have a self vs non-self recognition defect (10). This taken together with the facts that the adjacent stop and start codons overlap in the five other genes, *idsB, C, D, E* and *F* and that there is a 72-base pair (bp) non-coding region between *idsA* and *idsB*, suggest that *idsA* might be transcribed independently from the other genes (10, 17). We analyzed mRNA extracted from wild-type *P. mirabilis* strain BB2000 by reverse transcription-

polymerase chain reaction (RT-PCR) with primers that spanned adjacent genes. Our results showed that *idsA-F,* but not the genes flanking *idsA-F,* were on a single transcript. That is primers spanning intergenic regions between each gene in the *ids* cluster yielded PCR products of the predicted sizes. There was no detectable product with primers spanning the region between *idsA* and the gene upstream of it or *idsF* and the gene downstream, nor was there any product in reactions in which reverse transcriptase was omitted (data not shown).

It is nevertheless conceivable that there is a transcription start site in the region upstream of *idsA* and a secondary site in the intergenic region between *idsA* and *idsB*. To address this possibility we constructed a transcriptional reporter plasmid containing the 788-bp region between *idsA* and the adjacent upstream gene fused to *gfp* (pKG100). We also constructed a plasmid containing the *idsA-idsB* intergenic region (72 bp) and the first nine bp of *idsB* fused to *gfp* (pKG103). We introduced these plasmids into our wild-type *P. mirabilis* BB2000 and measured GFP expression. The plasmid containing the region upstream of *idsA* (pKG100), directed significant GFP expression, whereas pKG103 with *gfp* fused to the *idsA-idsB* intergenic region did not direct appreciable GFP expression (Fig. 1C). We conclude that expression of all of the *ids* genes is entirely dependent on a promoter or promoters upstream of *idsA*.

To examine the promoter region more closely, we constructed two plasmids: one with the complete 788-bp region upstream and the first nine basepairs of *idsA-gfp* (pKG102) and the other with only the proximal 435-bp region upstream and the first nine basepairs of *idsA* (pKG104). The GFP expression directed by each plasmid in *P. mirabilis* BB2000 was measured (Fig. 1D). Cells grown in broth (i.e. swimmer cells) showed similar levels of *idsA-gfp* expression from

either promoter construct. In swarms, higher levels of *idsA-gfp* expression occurred with the complete *idsA* upstream region as compared to the -435- +9 region (Fig. 1D). These data suggest the possibility of surface-contact dependent expression of the *P. mirabilis ids* operon but further work is required to address this question. Regardless, it appears that the minimal element for *idsA* transcription resides within a region extending 435 bp upstream of the *idsA* translation start site.

Bimodal and density-correlated expression of the *ids* operon. Examination of the edge of an advancing swarm of *P. mirabilis* containing pKG100 revealed a mixture of bright and dark individual cells (Fig. 2A). Cells at the advancing edge of a swarm tended to move in small packs; both bright and dark cells were often in an individual pack. In contrast, dark cells were only rarely observed in swarms of *P. mirabilis* constitutively expressing *gfp*; a relatively consistent expression level of fluorescence was observed across the population (Fig. 2B). The two modes of fluorescence in the *P. mirabilis* (pKG100) swarms, bright and dark, suggest that in advancing swarms of a single strain, only a subset of cells expresses the *ids* genes and therefore is capable of sensing self. As such when two separate swarms of a single strain approach, only a subset of cells in either swarm is capable of sensing self and presumably initiating a merger with the apposing swarm.

For several reasons it proved difficult to obtain quantitative information on the bimodal distribution of bright and dark cells in swarming populations. Cells that are not in the leading edge of the swarm are irregularly shaped, cells move in packs and are closely aligned along each other, and the developmental stage at the edge of a swarm is variable (consolidation rings versus

advancing swarms). Therefore we sought to determine whether swimmer cells grown in broth exhibited two modes of fluorescence expression. In fact broth-grown cells did exhibit a bimodal pattern of *ids* gene expression (Fig. 3A). The relative abundance of dark and bright cells changed as culture density increased. At optical densities below 0.8 dark cells predominated, while above 0.8 bright cells were dominant (Fig. 3A and B). This shift towards a higher percentage of p*idsA-gfp* expressing cells at densities above 0.8 was reflected in overall culture fluorescence measurements. When normalized to optical density, culture fluorescence decreased after inoculation until the optical density reached above 0.4-0.5 and then fluorescence per unit cell mass increased (Fig. 3C). A similar relationship between culture density and *gfp* expression was observed when we examined cells containing pKG102, with *idsA* fused in-frame to *gfp*. Furthermore, expression of *gfp* in cells containing pKG104 with *idsA* -435 to +9-*gfp* showed bimodal and cell density-related expression patterns similar to those described for constructs with the complete 788 bp *idsA* upstream region (data not shown). As a control we show that in cells with a plasmid-borne constitutive *gfp* fluorescence do not exhibit bimodality (Fig. S1). Thus our evidence supports the view that *ids* gene expression is bimodal and cell-density dependent in broth-grown swimmer cells or in swarmer cells on an agar surface.

The influence of an approaching swarm on *ids* expression. We next sought to determine whether an approaching *P. mirabilis* swarm, either self or non-self, influenced expression of the *ids* operon by analyzing cellular fluorescence in the wild-type strain BB200 carrying the *idsA-gfp* expression vector pKG102 at swarm edges that interface with either BB2000 (self) or an *ids* deletion mutant (Δ*ids*) derived from BB2000 (non-self). In a first analysis we measured the fluorescence intensity of the brightest pixel in slices of 13 μM by 6.5 μm that extended across

the region where two swarms either merged or formed a boundary (Fig. 4A). As expected, when strain BB2000 with the *idsA-gfp* vector pKG102 approached self (BB2000 with the backbone plasmid) the brightest pixel in a slice decreased progressively. A similar pattern was observed when the *gfp*-tagged BB2000 approached non-self (the *ids* deletion strain) except that fluorescence was higher in every slice through the boundary and receding into the denser regions of the swarm of *gfp*-tagged cells. This higher level of fluorescence suggested that approach of and interaction with non-self somehow induced high-level *idsA* expression in at least a subset of cells. This induction may result from contact with a non-self cell that has migrated across the boundary, which we know is occurring from our previous work (10), and for which there is evidence in Figure 3A; we observed some slices with a bright pixel in the boundary and on the non-self side of the boundary.

We next wanted to obtain additional evidence that migration towards a swarm recognized as non-self enhanced expression of the *ids* operon and to ask whether this phenomenon might require contact between cells from the two advancing swarms. To do this we used six-well plates and a fluorescence plate reader. Wells contained agar on which swarms of the *gfp*-tagged BB2000 and swarms of either BB2000 or the *ids*-deletion derivative moved towards each other. In some wells we placed a piece of 0.22-μm filter standing on edge into the agar between the two swarms. The filters allowed diffusion of small soluble molecules but did not allow cell passage. Consistent with a previous report that cell-cell contact is required for boundary formation (4), boundaries formed between self and non-self swarms in wells without a filter but not between swarms divided by a filter. Without a dividing filter the fluorescence was significantly higher in wells with *gfp*-tagged BB2000 and the non-self *ids* deletion mutant than in wells with the *gfp*-

tagged BB2000 and BB2000 (Fig. 4B). The increased fluorescence due to interactions with non-self cells primarily occurred at the boundary, specifically at the swarm edge that directly interacted with the apposing swarm (Fig. 4C); here there was a three-fold increase in fluorescence when non-self cells were was present (Fig. 4D). These finding support the hypothesis that migration towards a swarm recognized as non-self enhances expression of the *ids* operon. Furthermore, in wells with a dividing filter, self or non-self did not differently influence the fluorescence (Fig. 4B). These findings indicate that cell-cell contact or a non-diffusible factor is not only required for boundary formation but is also required for the non-self stimulation of *ids* expression.

Discussion

An important step in developing an understanding of how the *P. mirabilis ids* genes specify self-identity involves learning about *ids* expression. Here we show that the *ids* genes are transcribed as a single unit. This includes *idsA*, which itself is not a required identity determinant (10). Transcription of the *ids* operon depends on a promoter upstream of *idsA* and the minimal promoter element resides within the adjacent 435 bp upstream of the predicted *idsA* translational start site. There may be additional regulatory sequences further upstream that are required for surface stimulation of *ids* gene expression (Fig. 1D). The question of whether *ids* transcription is stimulated by contact with an agar surface is interesting and deserves further study. We note that surface contact induces *P. mirabilis* swimmer cells to differentiate into swarmer cells, and this differentiation involves the master motility regulator FlhDC (9, 11).

When we examined *ids* gene expression at a cellular level by using *P. mirabilis* containing an *idsA-gfp* plasmid and fluorescence microscopy there was an obvious bimodal expression of *ids* promoter activity, which continued to be present after several passages (data not shown), suggesting that there might be a bistable switch controlling *ids* transcription (Figs. 2 and 3). This was true when we examined cells from broth-grown populations or cells swarming on agar plates. It remains to be determined if the readout from our plasmid encoded *idsA-gfp* construct reflects expression from the native chromosomal *ids* operon, but we have no reason to believe it does not.

Our data also indicate that *ids* expression increases when broth cultures grow past a critical density (Fig. 3), and visual analysis suggests that there might be higher *ids* expression in more dense areas of the swarms (data not shown). How the apparent cell-density dependent and bistable expression of the *ids* operon are involved in self vs non-self discrimination is not clear. In considering these aspects of *ids* regulation it is important to remember that there are likely other as yet unidentified genes involved in recognition (10). It is also important to remember that all of our data on bistability and cell density-dependent expression come from studies of the *idsA* promoter on a plasmid we have introduced into *P. mirabilis*. Finally, it is prudent to keep in mind that in addition to formation of the boundaries between isogenic strains, which we have studied here, there can be proticine-dependent killing within the boundaries that form between two different clinical isolates of *P. mirabilis* (19). We have dissected out only one part of a complex set of behaviors occurring when swarms of two independent *P. mirabilis* isolates approach each other.

Given the limitations described in the previous paragraph there are nevertheless some interesting implications that can be drawn from our experiments. First, our evidence is consistent with the idea that there are more cells expressing the *ids* genes in the interior regions of a swarm where the cells are at high density in multiple layers than there are at the periphery of a swarm where cells exist as small monolayered packs. Perhaps bistable expression of *ids* genes in some way enables alternative responses in cells at the advancing edge of a swarm. This idea is congruous with previously described functions of bistable switches; although not commonly encountered they are known to regulate a variety of developmental processes including *Bacillus subtilis* sporulation, competence and motility (12, 13, 20). Bistability is thought to aid in population bet-hedging against uncertain environments (21). One uncertainty for an advancing swarm is whether it will encounter another advancing swarm of foreigners that can compete for resources and probably has a killing capability through proticine production or whether it will encounter fresh nutritional resources.

We present evidence that *ids*-expressing cells can traverse an established boundary and co-mingle with cells in a foreign population (Fig. 4A). We also present evidence that there is a higher level of *ids* expression in cells approaching a foreign swarm than in cells approaching a swarm recognized as self (Fig. 4). Enhancement of *ids* expression by a foreign swarm also appears to be dependent upon cell-cell contact or contact with a location that has been traversed by a cell or cells from a foreign swarm (Fig. 4B). In fact, *P. mirabilis* leave visible tracks on the surface (Fig. 2). The enhanced *ids* expression in cells at the edge of a swarm boundary with a foreign swarm might be a response to foreigners that have traversed the boundary.

Though we believe this analysis is an important step in developing a comprehensive view of how the *ids* genes serve as a self vs non-self discrimination system and that it provides important information that will need to be integrated into a molecular model for self vs non-self discrimination in *P. mirabilis*, it also raises many more questions than it answers. Is the observed surface stimulation of *ids* gene expression important and what is the mechanism of stimulation? Is the increase in *ids* gene expression at high cell densities important for boundary formation? What is the mechanistic basis of the apparently bistable *ids*-expression switch and is this critical for self vs non-self swarm discrimination? Is cell-cell contact really required for development of boundaries, and if so how is the information of self vs non-self transferred from one cell to the other?

ACKNOWLEDGEMENTS

K.A.G. was supported by NIH training grant AI55396. The research was partially supported by USPHS grant GM-59026 to E.P.G.

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Figure Legends

Figure 1. *Proteus mirabilis* territorial boundaries, the *ids* locus, and transcription from the *idsA* promoter. (A) A swarm plate of the wild-type strain BB2000, the BB2000 *idsA-F* deletion mutant (Δ*ids*), BB2000 with the *idsA-gfp* vector pKG100, or with an empty vector. A visible boundary formed between swarms of wild type and the deletion mutant. Swarms of the wild type merged regardless of the presence of pKG100. The bar is 1 cm. (B) Organization of the *P. mirabilis idsA-F* locus. The arrows indicate open reading frames. The region between *idsA* and the diverging ORF is 1899 bp. The distance between the *idsA* and *idsB* ORFs is 73 bp. The distance between *idsF* and the downstream ORF is 180 bp. (C) Fluorescence of *P. mirabilis* cells containing the *idsA* promoter-*gfp* expression vector pKG100 or pKG104, which contains the *idsA-B* intergenic region with *gfp* fused to *idsB*. Cells were harvested at a culture density of 4-5. (D) *P. mirabilis* BB2000 containing the *idsA-gfp* vector pKG102, which contains 788-bp upstream of *idsA*, or pKG104, which contains only 435 bp upstream of *idsA* were grown either in broth or on swarm-agar plates. Broth-grown cells were obtained at a culture density of 4-5, and cells. Swarm cells were obtained from agar plates after 24 h (at this time active swarming had ceased). Cells were suspended in PBS, fluorescence and optical density were measured and fluorescence units are normalized to optical density. Bars indicate means with standard deviation. GFP fluorescence units are normalized to optical density and measured as described in the Materials and Methods.

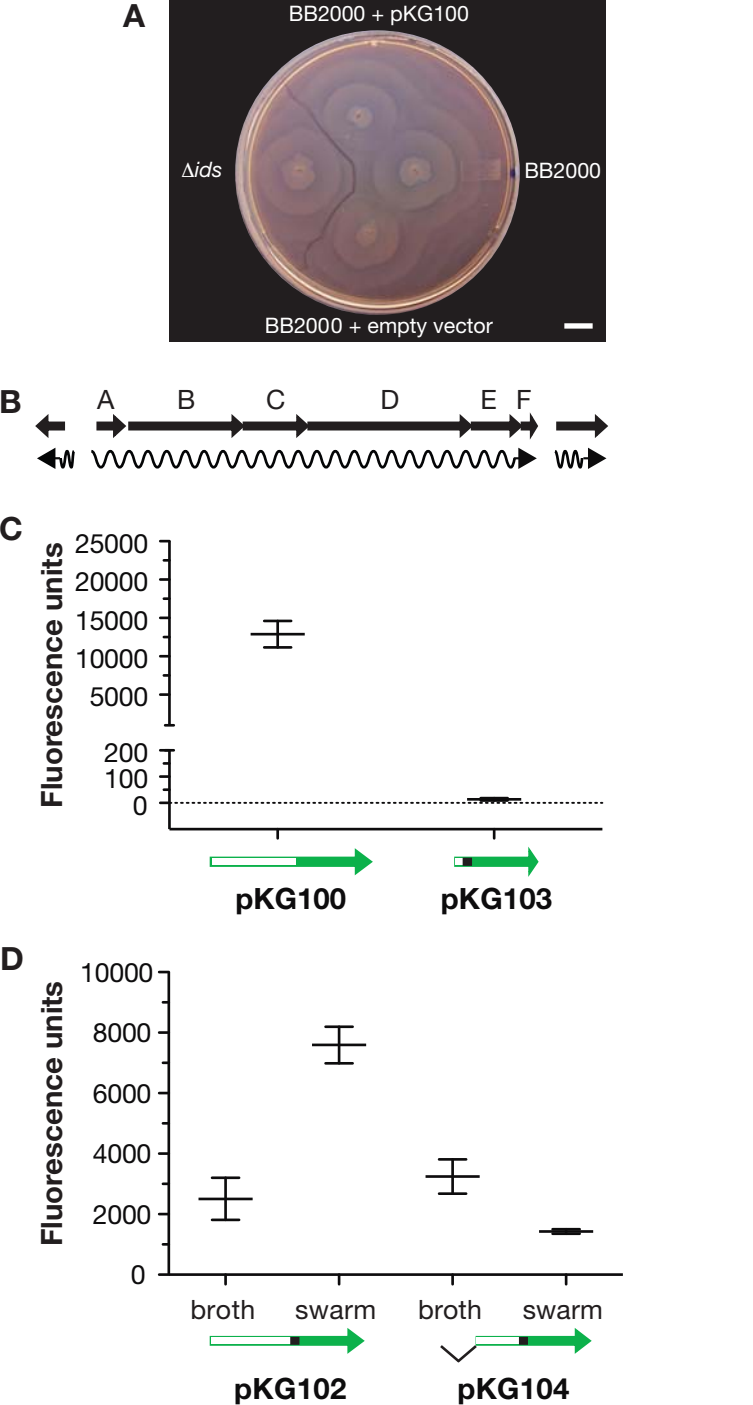
Figure 2. Migrating fronts of *P. mirabilis* swarms. (A) Bimodal expression of the pKG100 *idsA-gfp* fusion in cells of *P. mirabilis* at the outer edge of a swarm. Phase is on the left, fluorescence is in the center, and the false-colored image on the right shows the agar background in red,

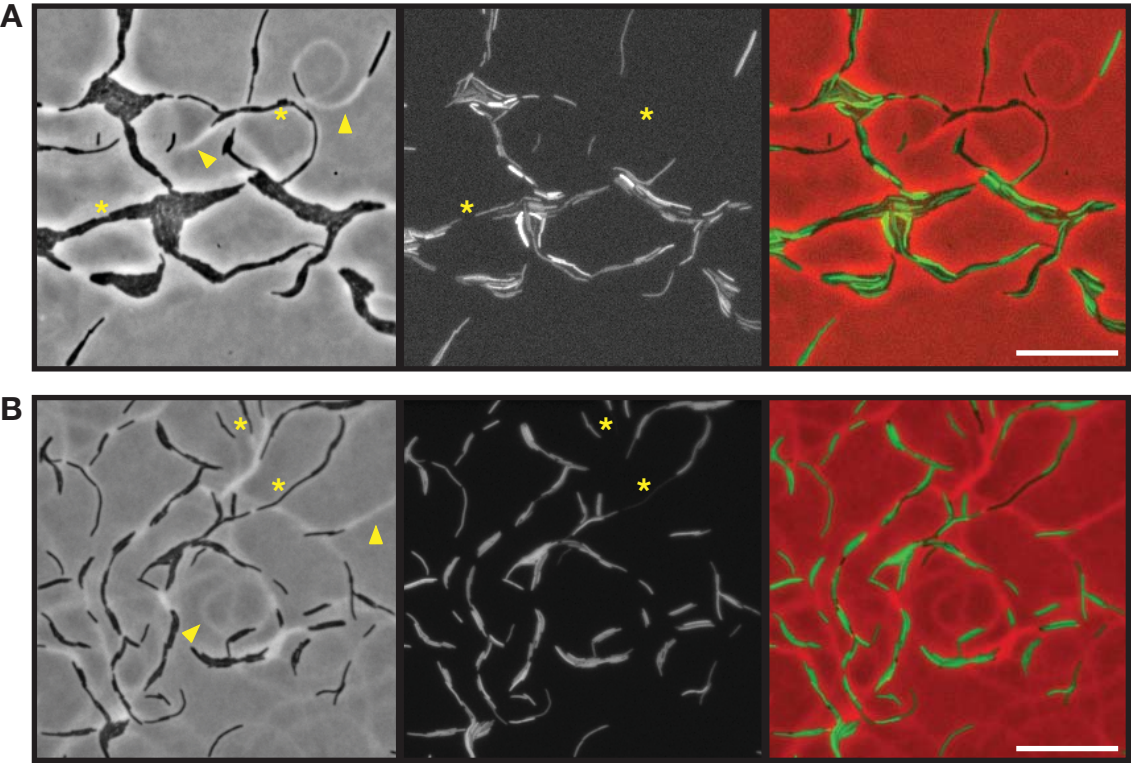
fluorescence bright cells in green and dark cells in black. Asterisks mark examples of dark cells; arrowheads indicate swarm tracks. (B) A control, the outer swarm edge of BB2000 cells carrying a chromosomally integrated constitutive *gfp* (10). Asterisks mark dim cells (dark cells were not evident); arrowheads indicate swarm tracks. The marker bars are 50 μm.

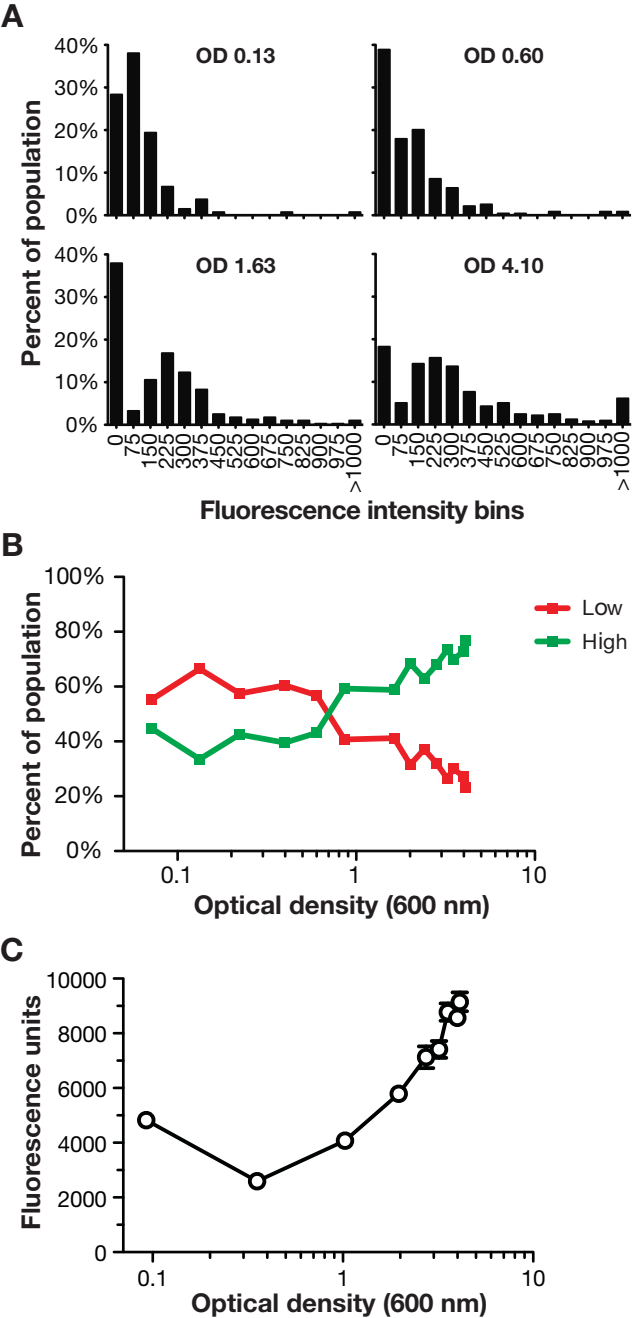
Figure 3. The *idsA* promoter shows a bimodal and culture density-dependent pattern of expression. (A) Histograms of individual cell fluorescence intensity in an LB-broth culture at different cell densities (OD at 600 nm). We measured fluorescence of 135 individual cells at OD 0.13, 255 cells at OD 0.60, 419 cells at OD 1.60, and 728 cells at OD 4.01. Cells with fluorescence levels below 100 are dark or very dim. (B) The percent of LB broth-grown *P. mirabilis* BB200 (pKG100) cells expressing *idsA-gfp* in relation to culture density. (C) Expression of *idsA* as a function of culture density in LB broth. Fluorescence units are normalized to optical density.

Figure 4. Effects of social recognition on *ids* expression. (A) Maximum fluorescence intensity averaged across swarm boundaries. The boundaries are between *P. mirabilis* BB2000 containing the *idsA-gfp* vector pKG102 and either BB2000 or the *ids* deletion mutant containing the vector control pKG101 (to allow antibiotic maintenance of the plasmids in all bacteria). We measured fluorescence in 0.65-μm by 13-μm intervals. The 0 position is in the swarm of BB2000 (pKG102) and 1500 is in the swarm of the vector-containing bacteria. The black box indicates the location where swarms either merged or where the boundary formed. Background fluorescence in dark colonies is about 200 units. (B) Total fluorescence of *P. mirabilis* BB2000 (pKG102). The swarms were approaching either BB2000 containing the vector control pKG101

or the *ids* deletion strain carrying pKG101. Where indicated a 0.22 μm filter divided the approaching swarms. The data are normalized to optical density. The boxes show ranges; the horizontal lines denote the means. There was a significant difference without the filter and no significant difference (NS) with the filter. (C) Fluorescence of *P. mirabilis* BB2000 (pKG102) calculated as in Fig. 4B. Fluorescence, normalized to optical density, was measured at discrete points along the swarm and was plotted such that the X-axis measures the distance from one edge of a well to the other. The swarms met between 1 cm and 2.2 cm from the edge of the well. Markers denote mean; bars are standard error of the mean. (D) The ratios (Δ*ids*:BB2000) of the data in Fig. 4C, plotted as a function of the distance from the edge of the well.


A
BB2000 + pKG100
Δids
BB2000
BB2000 + empty vector
B
A
B
C
D
E
F
C
Fluorescence units
25000
20000
15000
10000
5000
200
100
0
pKG100
pKG103
D
Fluorescence units
10000
8000
6000
4000
2000
0
broth
swarm
broth
swarm
pKG102
pKG104


A
B


A
OD 0.13
OD 0.60
OD 1.63
OD 4.10
Percent of population
40%
30%
20%
10%
0%
0
75
150
225
300
375
450
525
600
675
750
825
900
975
>1000
Fluorescence intensity bins
B
100%
80%
60%
40%
20%
0%
Low
High
0.1
1
10
Optical density (600 nm)
C
Fluorescence units
10000
8000
6000
4000
2000
0
0.1
1
10
Optical density (600 nm)

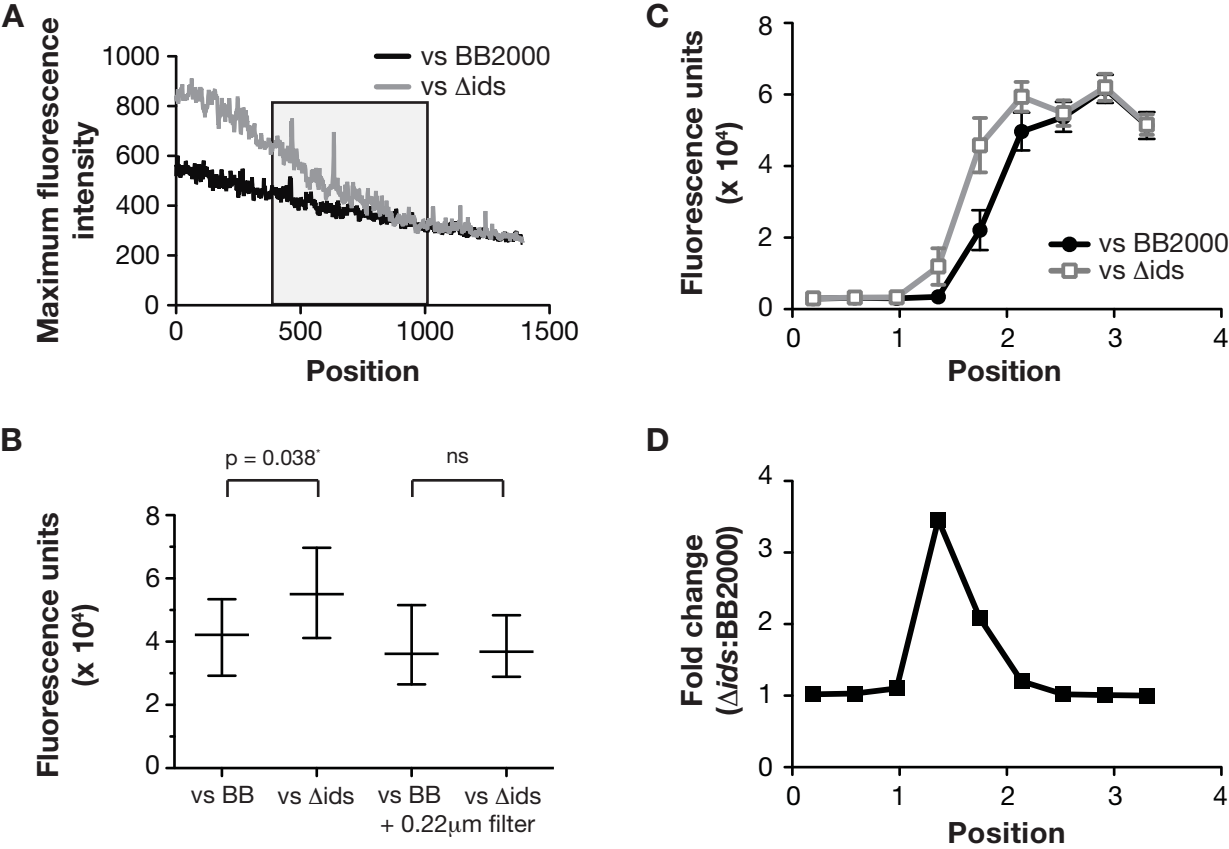
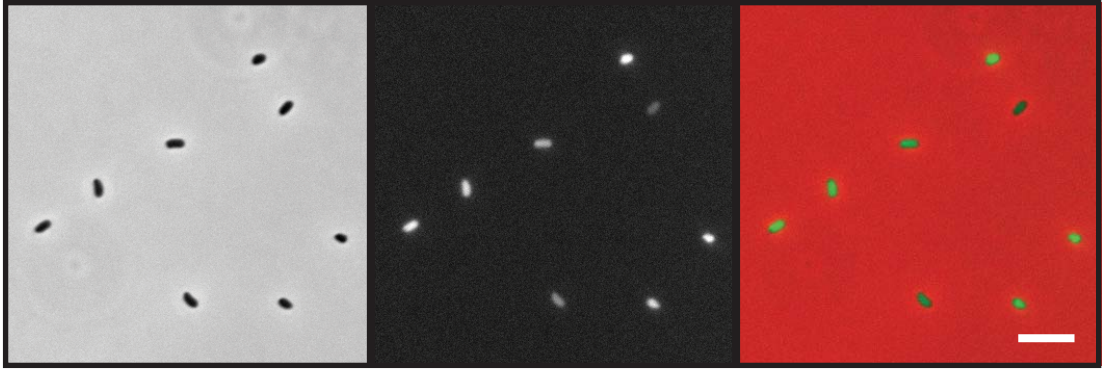
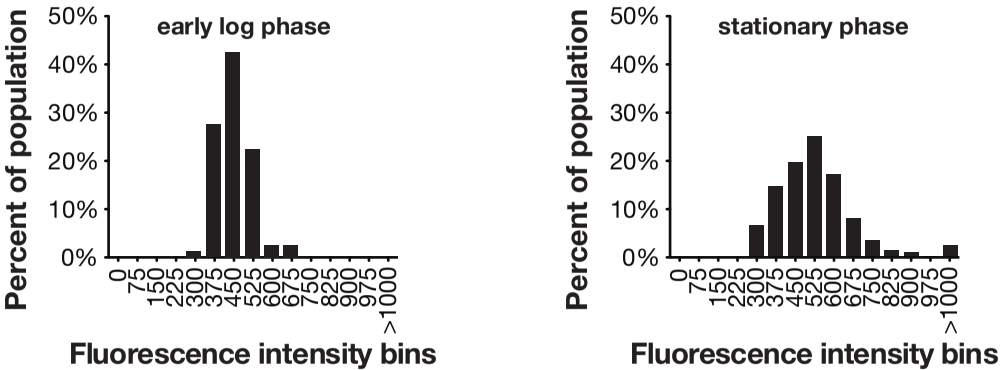

A
Maximum fluorescence intensity
1000
800
600
400
200
0
0
500
1000
1500
Position
vs BB2000
vs Δids
C
Fluorescence units (x 10⁴)
8
6
4
2
0
0
1
2
3
4
Position
vs BB2000
vs Δids
B
Fluorescence units (x 10⁴)
p = 0.038*
ns
8
6
4
2
0
vs BB
vs Δids
vs BB + 0.22μm filter
vs Δids
D
Fold change (Δids:BB2000)
4
3
2
1
0
0
1
2
3
4
Position

Figure S1. Normal distribution of fluorescence in cells of *P. mirabilis* BB2000 containing plasmid pKG105, which is a pKG101 derivative with *gfp* fused to tandem *lac* and *fla* promoters. (Top) From left to right, phase contrast, epifluorescence and false-colored (GFP fluorescence is green) microscope images. Cells were harvested during stationary phase and imaged as described in Materials and Methods. The marker bar is 5 μm. (Bottom) Histograms of individual cell fluorescence intensities in an LB-broth culture at early logarithmic phase (79 cells) and stationary phase (1633 cells). Both populations exhibited a normal distribution averaging around 450 to 525 arbitrary units. Cells with fluorescence levels below 100 are considered dark.

A



B


early log phase
Percent of population
50%
40%
30%
20%
10%
0%
0
75
150
225
300
375
450
525
600
675
750
825
900
975
>1000
Fluorescence intensity bins
stationary phase
Percent of population
50%
40%
30%
20%
10%
0%
0
75
150
225
300
375
450
525
600
675
750
825
900
975
>1000
Fluorescence intensity bins